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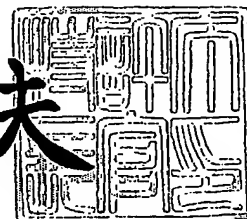Commissioner,
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【プルーフの要否】　　　要

【書類名】　　明細書

【発明の名称】　　４ｓ期神経芽細胞腫から単離された核酸

【特許請求の範囲】

　　【請求項１】　　配列表の配列番号１ないし１７４に記載の核酸配列からなる群より選ばれる１つの配列からなる核酸。

　　【請求項２】　　配列表の配列番号１ないし１４に記載の核酸配列からなる群より選ばれる１つの配列からなる、請求項１に記載の核酸。

　　【請求項３】　　請求項１または２に記載の核酸に相補的な核酸。

　　【請求項４】　　請求項１ないし３のいずれか１項に記載の核酸と、ストリンジェントな条件下でハイブリダイズする核酸。

　　【請求項５】　　以下の(a)或いは(b)の核酸を含む核酸プローブ：

(a)配列表の配列番号１ないし１７４に記載の核酸配列からなる群より選ばれる１つの配列の全長若しくは一部からなる核酸、またはそれに相補的な核酸；

(b)　配列表の配列番号１ないし１７４に記載の核酸配列からなる群より選ばれる１つの配列からなる核酸とストリンジェントな条件下でハイブリダイズする核酸、またはそれに相補的な核酸。

　　【請求項６】　　以下の(a)或いは(b)の核酸を含む請求項５に記載の核酸プローブ：

(a)配列表の配列番号１ないし１４に記載の核酸配列からなる群より選ばれる１つの配列の全長若しくは一部からなる核酸、若しくはそれに相補的な核酸；

(b)　配列表の配列番号１ないし１４に記載の核酸配列からなる群より選ばれる１つの配列からなる核酸とストリンジェントな条件下でハイブリダイズする核酸、若しくはそれに相補的な核酸。

　　【請求項７】　　請求項５または６に記載の核酸プローブを有効成分として含有する４ｓ期神経芽細胞腫の診断剤。

　　【請求項８】　　以下の(a)或いは(b)のＤＮＡを含むプライマー：

(a)配列表の配列番号１７５ないし１０７６に記載の核酸配列からなる群より選ばれる１つの配列からなるＤＮＡ、またはそれに相補的なＤＮＡ；

(b)配列表の配列番号１７５ないし１０７６に記載の核酸配列からなる群より選

ばれる１つの配列からなるＤＮＡとストリンジェントな条件下でハイブリダイズするＤＮＡ、またはそれに相補的なＤＮＡ。

【請求項９】　以下の(a)或いは(b)のＤＮＡを含むプライマー：

(a)配列表の配列番号１７５ないし２０２に記載の核酸配列、および配列番号５１９ないし５４０に記載の核酸配列からなる群より選ばれる１つの配列からなるＤＮＡ、若しくはそれに相補的なＤＮＡ、または配列表の配列番号７８５ないし７９８に記載の核酸配列からなる群より選ばれる１つの配列からなるＤＮＡ、若しくはそれに相補的なＤＮＡ；

(b)配列表の配列番号１７５ないし２０２に記載の核酸配列、および配列番号５１９ないし５４０に記載の核酸配列からなる群より選ばれる１つの配列からなるＤＮＡと、または配列表の配列番号７８５ないし７９８に記載の核酸配列からなる群より選ばれる１つの配列からなるＤＮＡとストリンジェントな条件下でハイブリダイズするＤＮＡ、若しくはそれに相補的なＤＮＡ。

【請求項１０】　請求項８または９に記載のプライマーを一組、有効成分として含有する４ｓ期神経芽細胞腫の診断キット。

【請求項１１】　神経芽細胞腫の臨床組織サンプルから配列表の配列番号１ないし１４に記載の核酸配列からなる群より選ばれる１つの配列からなる核酸の有無を検出することを特徴とする、４ｓ期神経芽細胞腫の判定方法。

【請求項１２】　固相支持体に、配列番号１ないし１７４に記載の核酸配列の全長若しくは一部からなる核酸を複数個組み合わせ、それらを固定してなる核酸マイクロアレイ。

【請求項１３】　固相支持体に、配列番号１７５ないし２０２に記載の核酸配列、配列番号５１９ないし５４０に記載の核酸配列、および配列番号７８５ないし７９８に記載の核酸配列からなる核酸を複数個組み合わせ、それらを固定してなる核酸マイクロアレイ。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】

本発明は、ヒト神経芽細胞腫において発現する遺伝子に由来する核酸類に関す

る。さらに詳しくは、本発明は、４ｓ期のヒト神経芽細胞腫において発現する遺伝子に由来する核酸類に関する。さらに、本発明は、このような核酸およびそれらの断片、あるいはそれらの組み合わせを利用した核酸プローブ、プライマーまたは核酸マイクロアレイ等からなる、４ｓ期神経芽細胞腫の診断剤および診断キット、さらには上記遺伝子からの核酸配列情報に基づく癌細胞のプログラム細胞死機構の解明に関する。

【０００２】

【従来の技術】

（腫瘍形成と遺伝子）

個々の腫瘍にはそれぞれの個性があり、発癌の基本的な原理は同じであっても、その生物学的特性は必ずしも同じではない。近年、癌の分子生物学や分子遺伝学が急速に進歩し、発癌やいわゆる腫瘍細胞のバイオロジーが遺伝子レベルで説明できるようになってきた。

【０００３】

（神経芽細胞腫）

神経芽細胞腫は末梢交感神経系細胞に由来する交感神経節細胞と副腎髄質細胞から発生する小児癌である。この交感神経系細胞は発生初期の神経堤細胞が腹側へ遊走し、いわゆる交感神経節が形成される場所で分化成熟したものである。その一部の細胞はさらに副腎部へ遊走し、先に形成されつつある副腎皮質を貫通して髄質部に達し、そこで髄質を形成する。神経堤細胞は、ほかの末梢神経細胞の起源ともなっており、後根神経節（知覚神経）、皮膚の色素細胞、甲状腺Ｃ細胞、肺細胞の一部、腸管神経節細胞などへ分化する。

【０００４】

（神経芽細胞腫の予後）

神経芽細胞腫は多彩な臨床像を示すことが特徴である（非特許文献１参照）。例えば、１歳未満で発症する神経芽細胞腫は非常に予後が良く、大部分が分化や細胞死を起こして自然退縮する（予後良好型ともいう）。現在、広く実施されている生後６か月時の尿のマススクリーニングで陽性となる神経芽細胞腫の多くは、この自然退縮を起こしやすいものに属する。一方、１歳以上で発症する神経芽

細胞腫は悪性度が高く、多くの場合、治療に抵抗して患児を死に至らしめる（予後不良型ともいう）。１歳以上の悪性度の高い神経芽細胞腫は、体細胞突然変異（Somatic mutation）が起こり、モノクローナルであるのに対し、自然退縮する神経芽細胞腫では生殖細胞突然変異（germ line mutation）のみの遺伝子変異でとどまっているとの仮説もある（非特許文献２参照）。さらに、臨床的にこれらの型の中間に位置する中間型の神経芽細胞腫もある。

【０００５】

腫瘍の進行度からこれら神経芽細胞腫を分類すると以下のようになる。

１期：副腎または交感神経節に原発し、限局している。

２期：原発巣に限局した腫瘍と局部リンパ節転移のみを有する。リンパ節転移は正中線を越えない。

３期：腫瘍が正中線を越えて対側に浸潤またはリンパ節転移をきたす。

４期：骨、骨髄、眼窩部に遠隔転移を起こす。

４ｓ期：１歳未満に発症し、骨髄、皮膚、肝に遠隔転移する。

【０００６】

予後良好型の神経芽細胞腫は、１、２、４ｓ期の腫瘍であり、予後不良型および中間型の神経芽細胞腫は、３、４期の腫瘍である。４ｓ期の腫瘍は、特異的であり、通常生後数ヶ月の乳児に発症し、急速に腫瘍が増殖転移するが、突然増殖が止まり、その後は自然に腫瘍が消失する。このように、自然退縮する腫瘍と悪性増殖する腫瘍との間の違いは、発症年齢と転移部位、さらに進行度が明らかに異なる。

【０００７】

（神経芽細胞腫の予後を推定する遺伝子）

最近の分子生物学的研究の進展により、神経成長因子（nerve growth factor：ＮＧＦ）の高親和性レセプターであるＴｒｋＡの発現が分化と細胞死の制御に深くかかわっていることが明らかとなってきた（非特許文献３参照）。Ｔｒｋは神経栄養因子の高親和性受容体で、膜貫通型受容体であり、Ｔｒｋ－Ａ、Ｂ、Ｃの３つが主なものである。

【０００８】

　　Ｔｒｋファミリー受容体は、中枢神経および末梢神経系において、特異的な神経細胞の分化と生存維持に重要な役割を果たしている（非特許文献４参照）。腫瘍細胞の生存や分化はＴｒｋチロシンキナーゼやＲｅｔチロシンキナーゼからのシグナルで制御されている。なかでも、ＴｒｋＡ受容体の役割は最も重要で、予後良好型の神経芽細胞腫ではＴｒｋＡの発現が著しく高く、これからのシグナルが腫瘍細胞の生存・分化、または細胞死（アポトーシス）を強く制御している。一方、予後不良型の神経芽細胞腫では、ＴｒｋＡの発現が著しく抑えられており、これに代わってＴｒｋＢあるいはＲｅｔからのシグナルが生存の促進という形で腫瘍の進展を助長している。

【０００９】

　　また、神経の癌遺伝子であるN-mycの増幅が神経芽細胞腫の予後に関連していることも明らかになってきた（非特許文献５参照）。この遺伝子は神経芽細胞腫で初めてクローニングされたが、正常細胞や予後良好型の神経芽細胞腫では通常１倍体当たり１つしか存在しないのに対し、予後不良型の神経芽細胞腫においては数十倍に増幅されているのが見つかった。

【００１０】

　　上記の遺伝子以外にも、予後良好型の神経芽細胞腫で高発現する遺伝子として、ＣＤ４４、ＰＴＮ、caspase等が知られており、また予後不良型の神経芽細胞腫で高発現する遺伝子としては、ＳＶＶ（survivin）、ＭＫ（midkine）等が知られている。

【００１１】

　　さらに、本発明者らは、予後良好型の神経芽細胞腫において、一群の新規な遺伝子が高発現していることを見出し（特許文献１参照）、また対照的に予後不良型の神経芽細胞腫において、別の一群の新規な遺伝子が高発現していることを見出した（特許文献２参照）。

【００１２】

　　【特許文献１】

　　　国際公開ＰＣＴ／ＪＰ０１６３１号パンフレット

　　【特許文献２】

国際公開ＰＣＴ／ＪＰ０１６２９号パンフレット

【０１３】

【非特許文献１】

中川原，「神経芽腫の発生とその分子機構」，小児内科，１９９８年，第３０巻，ｐ．１４３

【非特許文献２】

ヌーソン・エー・ジーら（Knudson AG et al.），「４ｓ期神経芽細胞腫の退縮－遺伝学的仮説（Regression of neuroblastoma IV-S:A genetic hypothesis）」，ニューイングランド・ジャーナル・オブ・メディスン（N. Engl. J. Med.），米国，１９８０年，第３０２巻，ｐ．１２５４

【非特許文献３】

ナカガワラ・エー（Nakagawara A.），「ＮＧＦそして神経芽細胞腫（The NGF story and neuroblastoma）」，メディカル・ペディアトリック・オンコロジー（Med. Pediatr. Oncol.），米国，１９９８年，第３１巻，ｐ．１１３

【非特許文献４】

中川原等，「神経芽細胞腫におけるニューロトロフィン受容体の発現と予後」，小児外科，１９９７年，第２９巻，ｐ．４２５－４３２

【非特許文献５】

中川原，「脳・神経腫瘍の多段階発癌」，モレキュラー・メディスン（Molecular Medicine），１９９９年，第３６４巻，ｐ．３６６

【００１４】

【発明が解決しようとする課題】

しかしながら、現在までに４ｓ期神経芽細胞腫において発現する（特に、特異的に）遺伝子についてはほとんど知られていなかった。さらに、上記のように４ｓ期神経芽細胞腫は自然退縮するので、この原因となる遺伝子の同定も急務である。

【００１５】

本発明は、上記従来技術の有する課題に鑑みてなされたものであり、一般的に

神経芽細胞腫の予後良不良に関係する遺伝子の核酸配列を明らかにし、そのような遺伝子情報の提供および予後良不良に関する診断を可能とすることを目的とする。本発明は、特定的には神経芽細胞腫の予後を診断し、該細胞腫の進行度分類を行い、４ｓ期神経芽細胞腫の判定を可能とすることを目的とする。

【００１６】

【課題を解決するための手段】

本発明者らは鋭意研究した結果、ヒト神経芽細胞腫の予後を検定し、予後良好型および予後不良型の臨床組織の各々からｃＤＮＡライブラリーを作製することに成功した。これら２種類のｃＤＮＡライブラリーから各々約２４００個のクローンをクローニングし、神経芽細胞腫の予後の良悪によって分類し、それぞれのサブセットで遺伝子のプロファイリングを行った。

【００１７】

そこで本発明者らは、前記サブセット間で示差的に発現し、かつ予後良好型の臨床組織でのみ発現が増強している遺伝子群を見いだした。加えて、本発明者は、予後不良型の臨床組織でのみ発現が増強している遺伝子群をも見いだした。かかる知見に基づき、本発明者は少なくとも予後良好型の臨床組織または、予後不良型の臨床組織でのみ発現が増強している遺伝子を検出およびクローニングするための核酸配列情報を提供することを可能とした。

【００１８】

さらに、本発明者らは、４ｓ期神経芽細胞腫の臨床組織から同様にｃＤＮＡライブラリーを作製することに成功した。このライブラリーから約２７００個のクローンをクローニングした。このライブラリーのサブセットと、予後良好型および予後不良型の臨床組織からのライブラリーのサブセットを解析して、これらのサブセット間で発現する約１６００個の遺伝子のプロファイリングを行った。その結果、前記サブセット間で示差的に発現する４５２個の遺伝子を同定した。これらの遺伝子をシークエンスしたところ、３０８個の新規な遺伝子と、残り１４４個の既知の遺伝子とから成っていた。前記遺伝子をそれぞれのサブセット間での発現パターンに従って、分類し７つの群にグループ化した。

【００１９】

　かかる知見に基づき、本発明者らは、４ｓ期神経芽細胞腫を特徴づける発現パターンを呈する遺伝子を検出およびクローニングするための遺伝子情報（核酸配列情報等）を提供することを可能とした。さらに該核酸配列情報に基づき、神経芽細胞腫の予後診断法（特に、進行度分類）を、４ｓ期神経芽細胞腫の判定を含めて、可能とする診断剤や診断キットを提供することを可能とし、本発明を完成した。

【００２０】

　すなわち、本発明によれば、

配列表の配列番号１ないし１７４に記載の核酸配列からなる群より選ばれる１つの配列からなる核酸が提供される。

【００２１】

　好ましい核酸は、前記配列番号１ないし１７４のうち、配列番号１ないし１４のいずれか一つに記載の核酸配列からなる核酸である。

【００２２】

　また、本発明によれば、上記これらの核酸に相補的な核酸も提供される。

【００２３】

　また、本発明によれば、上記の核酸と、またはそれに相補的な核酸とストリンジェントな条件下でハイブリダイズする核酸が提供される。

また、本発明によれば、

以下の(a)或いは(b)の核酸を含む核酸プローブが提供される：

(a)配列表の配列番号１ないし１７４に記載の核酸配列からなる群より選ばれる１つの配列の全長若しくは一部からなる核酸、またはそれに相補的な核酸；

(b)配列表の配列番号１ないし１７４に記載の核酸配列からなる群より選ばれる１つの配列からなる核酸とストリンジェントな条件下でハイブリダイズする核酸、またはそれに相補的な核酸。

【００２４】

　好ましくは、前記(a)或いは(b)の核酸がＤＮＡである。

【００２５】

　また、好ましくは、前記(a)または(b)の核酸が配列番号１ないし１４に記載の

核酸配列からなる群より選ばれる１つの配列からなる核酸である。

【００２６】

また、本発明によれば上記の核酸プローブを有効成分として含有する４ｓ期神経芽細胞腫の診断剤が提供される。

【００２７】

さらに、本発明によれば、

以下の(a)或いは(b)のＤＮＡを含むプライマーが提供される：

(a)配列表の配列番号１７５ないし１０７６に記載の核酸配列からなる群より選ばれる１つの配列からなるＤＮＡ、またはそれに相補的なＤＮＡ；

(b)配列表の配列番号１７５ないし１０７６に記載の核酸配列からなる群より選ばれる１つの配列からなるＤＮＡとストリンジェントな条件下でハイブリダイズするＤＮＡ、またはそれに相補的なＤＮＡ。

【００２８】

好ましくは、前記(a)或いは(b)のＤＮＡが配列番号１７５ないし２０２に記載の核酸配列、および配列番号５１９ないし５４０に記載の核酸配列からなる群より選ばれる１つの配列からなるＤＮＡ、または配列表の配列番号７８５ないし７９８に記載の核酸配列からなる群より選ばれる１つの配列からなるＤＮＡである。

【００２９】

また、本発明によれば上記のプライマーを一組、有効成分として含有する４ｓ期神経芽細胞腫の診断キットが提供される。

【００３０】

また、本発明によれば神経芽細胞腫の臨床組織サンプルから配列表の配列番号１ないし１４に記載の核酸配列からなる群より選ばれる１つの配列からなる核酸の有無を検出することを特徴とする、４ｓ期神経芽細胞腫の判定方法が提供される。

【００３１】

加えて、本発明によれば固相支持体に、配列表の配列番号１ないし１７４に記載の核酸配列からなる核酸の全長若しくは一部からなる核酸を複数個組み合わせ

て、固定してなる核酸マイクロアレイが提供される。

【００３２】

また、本発明によれば固相支持体に、配列番号１７５ないし２０２に記載の核酸配列、配列番号５１９ないし５４０に記載の核酸配列、および配列番号７８５ないし７９８に記載の核酸配列からなる核酸を複数個組み合わせ、それらを固定してなる核酸マイクロアレイが提供される。ここで、記載された配列番号を有する核酸配列からなる核酸の複数個の任意の組み合わせが用いられる。

【００３３】

【発明の実施の形態】

以下、本発明に係る神経芽細胞腫に発現する遺伝子（以下、「本発明の遺伝子」という）に由来する核酸（以下、「本発明の核酸」という）について、その用途を含めて、本発明の好適な実施の形態を参照して、詳細に説明する。

【００３４】

本発明の核酸は、上述のごとく本発明の遺伝子に由来するものであり、該遺伝子を構成するか或いは該遺伝子からインビボまたはインビトロの過程によって得られる。該核酸の鎖長には特に制限はなく、本明細書では前記遺伝子の一部に対応する核酸断片を含めて「本発明の核酸」という。核酸の鎖長が短い場合、その核酸は化学的手法で合成することができる。

【００３５】

本明細書で使用する「核酸」という用語は、例えばＤＮＡまたはＲＮＡ、或いはそれらから誘導された活性なＤＮＡ若しくはＲＮＡでありうるポリヌクレオチドを指し、好ましくは、ＤＮＡまたはＲＮＡを意味する。特に好ましい核酸は、本明細書中に開示されるヒトｃＤＮＡ配列と同一か、またはそれに相補的な配列を有する。

【００３６】

また、本発明で使用する「ストリンジェントな条件下でハイブリダイズする」という用語は、２つの核酸（または断片）が、サムブルックら（Sambrook, J.）の「大腸菌におけるクローン遺伝子の発現（Expression of cloned genes in E. coli）」，モレキュラー・クローニング：ア・ラボラトリー・マニュアル（Mol

ecular Cloning: A laboratory manual)，米国，コールド・スプリング・ハー
バー・ラボラトリー・プレス（Cold Spring Harbor Laboratory Press），１９
８９年，p．９．４７-９．６２，p．１１．４５-１１．６１に記載されたハイ
ブリダイゼーション条件下で、相互にハイブリダイズすることを意味する。

【００３７】

より具体的には、前記「ストリンジェントな条件」とは、約４５℃において６
．０×ＳＳＣでハイブリダイゼーションを行った後に、５０℃で２．０×ＳＳＣ
で洗浄することを指す。ストリンジェンシーの選択のため、洗浄工程における塩
濃度を、例えば低ストリンジェンシーとしての約２．０×ＳＳＣ、５０℃から、
高ストリンジェンシーとしての約０．２×ＳＳＣ、５０℃まで選択することがで
きる。さらに、洗浄工程の温度を低ストリンジェエンシー条件の室温、約２２℃
から、高ストリンジェンシー条件の約６５℃まで高くすることができる。

【００３８】

また、本明細書で使用する「核酸」という用語は、単離された核酸を指し、こ
れは組換えＤＮＡ技術により調製された場合は細胞物質、培養培地を実質的に含
有せず、化学合成された場合には前駆体化学物質またはその他の化学物質を実質
的に含まない、核酸またはポリペプチドを指す。

【００３９】

本明細書で使用する「予後良好型」とは、ヒト神経芽細胞腫のうち、腫瘍が限
局して存在するか、または退縮や良性の交感神経節細胞腫になった状態を指し、
Ｎ－ｍｙｃその他腫瘍マーカー（ＴｒｋＡ、染色体異常等）から判断して、悪性
度が低いと医師によって判断されるものである。本発明の好適な実施の形態では
、病期１または２、発症年齢が１歳未満、手術後５年以上再発なく生存し、臨床
組織中にＮ－ｍｙｃの増幅が認められないものを予後良好型としたが、このよう
な特定の例には限定されない。また、本明細書で使用する「予後不良型」とは、
ヒト神経芽細胞腫のうち、腫瘍の進行が認められる状態を指し、Ｎ－ｍｙｃその
他腫瘍マーカーから判断して、悪性度が高いと医師によって判断されるものであ
る。本発明の好適な実施の形態では、病期４、発症年齢が１歳以上、手術後３年
以内に死亡、臨床組織中にＮ－ｍｙｃの増幅が認められたものを予後不良型とし

たが、このような特定の例には限定されない。

【００４０】

なお、４ｓ期神経芽細胞腫は、上記のような臨床分子生物学的分類に従えば「予後良好型」に分類されるが、本明細書中では便宜上、「予後良好型」とは区別して取り扱う。

【００４１】

神経芽細胞腫は、ヒトでは２種類しか知られていない神経細胞そのものの腫瘍の１つであり、そこで発現している遺伝子を解析することは、神経細胞のバイオロジーを理解する上で非常に有用な知見をもたらすものと考えられる。すなわち、脳や末梢神経から、部位特異的な均質な組織を得ることは極めて困難で、事実上不可能である。一方、神経芽細胞腫は、末梢交感神経細胞に由来するほぼ均一な神経細胞集団（腫瘍化してはいるが）から成り、均質に発現している神経関連遺伝子が得られる可能性が高い。また、神経芽細胞腫は癌であるため、神経発生の未熟な段階で発現している重要な遺伝子が多いことも特徴として挙げられる。

【００４２】

さらに、神経芽細胞腫は、予後の良好なものと予後の不良なものとが臨床的、生物学的に明瞭に区別される。予後良好型の神経芽細胞腫の癌細胞は、増殖速度が極めて遅く、ある時点から自然退縮を始めることが特徴である。これまでの知見から、この自然退縮では、神経細胞の分化およびアポトーシス（神経細胞死）が起こっており、正常神経細胞の成熟段階で起こる分化とプログラム細胞死と非常によく似た現象であることが分かってきた。従って、この腫瘍で発現している遺伝子を解析することによって、神経の分化やアポトーシスに関連した重要な遺伝子情報を入手できる可能性が極めて高い。

【００４３】

上記の有用な遺伝子情報を入手できる遺伝子である本発明の遺伝子およびそれらに由来する本発明の核酸は、４ｓ期神経芽細胞腫の臨床組織（以下、４ｓとも略称する）に見出されたものであるが、予後良好型の臨床組織（以下、"Ｆ（favorable）"とも略称する）および予後不良型の臨床組織（以下、"ＵＦ（unfavorable）"とも略称する）でのそれら遺伝子の発現を比較すると以下のような特徴を

有する。

【００４４】

　すなわち、前述のようにして得られ、少なくとも部分的にシークエンスした４５２個の遺伝子をそれぞれのサブセット間での発現パターンに基づいて、分類し７つの群にグループ化したところ、次のようになる。

【００４５】

（グループＩ）

　このグループに属する遺伝子は、その発現（４ｓ）がＵＦと同程度であり、Ｆより低い。さらに、これら遺伝子をサブグループに分類すると、Ｉ－１、Ｉ－２およびＩ－３となる。各サブグループの遺伝子発現パターンについては、表１を参照。

【００４６】

　Ｉ－１に属する特定のクローンは、nbla20026（配列番号171），nbla20421（配列番号172），nbla22298（配列番号173），nbla22549（配列番号174）およびnbla23020（以上、新規遺伝子）である。

【００４７】

　Ｉ－２に属する特定のクローンは、nbla20113，nbla20146（配列番号137），nbla20170（配列番号138），nbla20216（配列番号139），nbla20253，nbla20549，nbla20657（配列番号140），nbla20688（配列番号141），nbla20755（配列番号142），nbla20835，nbla20968，nbla21013（配列番号143），nbla21087，nbla21172（配列番号144），nbla21189，nbla21200（配列番号145），nbla21214，nbla21255（配列番号146），nbla21337，nbla21344，nbla21345（配列番号147），nbla21410（配列番号148），nbla21522（配列番号149），nbla21631（配列番号150），nbla21788（配列番号151），nbla21897（配列番号152），nbla21956，nbla22116（配列番号153），nbla22223（配列番号154），nbla22228，nbla22344（配列番号155），nbla22351，nbla22361，nbla22474，nbla22629，nbla22939（配列番号156），nbla23084（配列番号157），nbla23103（配列番号158），nbla23234（配列番号159），nbla23300（配列番号160），nbla23369（配列番号161），nbla23436（配列番号162），nbla23511（配列番号163），nbla23664（配列番号164），nbla23775，nbla23860（配列番号165），nbla23877（配列番号

166），nbla23998(配列番号167)，nbla24043(配列番号168)，nbla24182，nbla242

85，nbla24402(配列番号169)，nbla24434，nbla24460，nbla24762，nbla24821(

配列番号170)，nbla24893，nbla24973，nbla24986（以上、新規遺伝子）、nbla2

0279，nbla20687，nbla20924，nbla21168，nbla21303，nbla21483，nbla21838，

nbla21917，nbla22099，nbla22438，nbla23111，nbla23208，nbla24118，nbla24

279，nbla24771および nbla24871（以上、既知遺伝子）である。

【００４８】

　Ｉ－３に属する特定のクローンは、nbla20084(配列番号129)，nbla21081(配列

番号130)，nbla21420(配列番号131)，nbla21761，nbla22452(配列番号132)，nbl

a22595(配列番号133)，nbla22676(配列番号134)，nbla22909(配列番号135)，nbl

a23456，nbla24297，nbla24435(配列番号136)，nbla24719（以上、新規遺伝子)

、nbla20117，nbla20238，nbla20904，nbla23293，nbla23297，nbla23311，nbla

23589，nbla23629，nbla23862，nbla24133およびnbla24761（以上、既知遺伝子

）である。

【００４９】

（グループII）

　このグループに属する遺伝子は、その発現（４ｓ）がＦと同程度であり、ＵＦ

より高い。さらに、これら遺伝子をサブグループに分類すると、II－１、II－２

およびII－３となる。各サブグループの遺伝子発現パターンについては、表１を

参照。

【００５０】

　II－１に属する特定のクローンは、nbla20365(配列番号117)，nbla20378(配列

番号118)，nbla20511(配列番号119)，nbla21039(配列番号120)，nbla21107(配列

番号121)，nbla21367(配列番号122)，nbla21790(配列番号123)，nbla21855，nbl

a22253(配列番号124)，nbla22355(配列番号125)，nbla22704，nbla22832(配列番

号126)，nbla23394，nbla23512，nbla23755(配列番号127)，nbla24084，nbla243

76，nbla24549(配列番号128)（以上、新規遺伝子）、nbla20624，nbla22029，nb

la22424，nbla22594およびnbla22622（以上、既知遺伝子）である。

【００５１】

　II－２に属する特定のクローンは、nbla20001（配列番号58），nbla20083（配列番号59），nbla20125，nbla20182（配列番号60），nbla20231，nbla20248（配列番号61），nbla20250（配列番号62），nbla20268，nbla20330（配列番号63），nbla20395，nbla23973，nbla23983（配列番号64），nbla24041，nbla24082，nbla24104，nbla24111（配列番号65），nbla24142（配列番号66），nbla24157（配列番号67），nbla24230（配列番号68），nbla24239，nbla20541（配列番号69），nbla20555（配列番号70），nbla20638，nbla20645（配列番号71），nbla20713（配列番号72），nbla20765，nbla20789，nbla20792，nbla20798，nbla21024，nbla24250（配列番号73），nbla24254（配列番号74），nbla24327（配列番号75），nbla24363，nbla24510（配列番号76），nbla24554（配列番号77），nbla24604（配列番号78），nbla24622，nbla24646，nbla24672，nbla21037（配列番号79），nbla21077，nbla21089，nbla21130，nbla21161（配列番号80），nbla21170（配列番号81），nbla21198（配列番号82），nbla21266，nbla21298（配列番号83），nbla21379（配列番号84），nbla24705（配列番号85），nbla24709，nbla24748，nbla24831，nbla24972，nbla21385（配列番号86），nbla21413，nbla21416（配列番号87），nbla21520，nbla21599（配列番号88），nbla21681（配列番号89），nbla21878（配列番号90），nbla21922（配列番号91），nbla21936，nbla22004-2（配列番号92），nbla22004-1（配列番号93），nbla22028，nbla22085（配列番号94），nbla22093，nbla22119（配列番号95），nbla22149（配列番号96），nbla22161（配列番号97），nbla22218，nbla22252（配列番号98），nbla22347（配列番号99），nbla22352（配列番号100），nbla22394（配列番号101），nbla22423（配列番号102），nbla22439（配列番号103），nbla22451，nbla22455，nbla22464，nbla22465，nbla22487，nbla22633（配列番号104），nbla22669，nbla22698（配列番号105），nbla22726，nbla22886，nbla22896（配列番号106），nbla23012，nbla23038，nbla23167（配列番号107），nbla23339（配列番号108），nbla23352（配列番号109），nbla23575（配列番号110），23592（配列番号111），nbla23601（配列番号112），nbla23630（配列番号113），nbla23718，nbla23719，nbla23754（配列番号114），nbla23892（配列番号115），nbla23951，nbla23956（配列番号116）（以上、新規遺伝子）、nbla20393，nbla20423，nbla20510，nbla20833，nbla20931，nbla20943，nbla21258，nbla21268，nbla21273，nbla21412，nbla21578，nbla21614，nbla21624，nbla2165

5, nbla21670, nbla21787, nbla21954, nbla21979, nbla22043, nbla22137, nbl a22192, nbla22325, nbla22327, nbla22337, nbla22482, nbla22763, nbla22788, nbla22839, nbla22851, nbla22935, nbla22937, nbla23238, nbla23327, nbla 23360, nbla23519, nbla23553, nbla23554, nbla23683, nbla23812, nbla23823, nbla23849, nbla23882, nbla23910, nbla24064, nbla24405, nbla24897およびn bla24913（以上、既知遺伝子）である。

【００５２】

II−３に属する特定のクローンは、nbla20134, nbla20181, nbla20264（配列番号31）, nbla20269（配列番号32）, nbla20276, nbla20406（配列番号33）, nbla2070 9, nbla20782, nbla20788, nbla20949（配列番号34）, nbla21046, nbla21122, nb la21211, nbla21233, nbla21251（配列番号35）, nbla21334（配列番号36）, nbla21 356（配列番号37）, nbla21375, nbla21418（配列番号38）, nbla21480（配列番号39）, nbla21509（配列番号40）, nbla21524, nbla21527（配列番号41）, nbla21551（配列番号42）, nbla21735（配列番号43）, nbla21843, nbla21934, nbla22153, nbla2 2247（配列番号44）, nbla22382, nbla22477（配列番号45）, nbla22571, nbla22639（配列番号46）, nbla22789, nbla23060, nbla23174（配列番号47）, nbla23198（配列番号48）, nbla23218, nbla23328（配列番号49）, nbla23420（配列番号50）, nbla 23483（配列番号51）, nbla23545, nbla23653, nbla23666, nbla23760, nbla23808（配列番号52）, nbla23830, nbla23851（配列番号53）, nbla23942, nbla24011（配列番号54）, nbla24131, nbla24235（配列番号55）, nbla24556（配列番号56）, nbla 24800（配列番号57）, nbla24908 （以上、新規遺伝子） 、nbla20133, nbla20263, nbla20723, nbla20748, nbla20915, nbla21016, nbla21034, nbla21067, nbla21 167, nbla21319, nbla21331, nbla21516, nbla21682, nbla21691, nbla21822, n bla21976-2, nbla21977, nbla22159, nbla22168, 22215-1, nbla22244, nbla222 63, nbla22548, nbla23033, nbla23231, nbla23284, nbla23329-1, nbla23384, nbla23556, nbla23674, nbla23879-2, nbla24098, nbla24329, nbla24334, nbla 24439-1, nbla24443, nbla24507, nbla24836, nbla24958およびnbla24989 （以上、既知遺伝子） である。

【００５３】

（グループIII）

このグループに属する遺伝子は、その発現（４ｓ）がＦと同程度であり、ＵＦより低い。さらに、これら遺伝子をサブグループに分類すると、III－１、III－２およびIII－３となる。各サブグループの遺伝子発現パターンについては、表１を参照。

【００５４】

III－１に属する特定のクローンは、nbla20874（新規遺伝子）およびnbla23262（既知遺伝子）である。

【００５５】

III－２に属する特定のクローンは、nbla20604，nbla21226，nbla21908（配列番号27），nbla21928，nbla22027（配列番号28），nbla22082（配列番号29），nbla22643，nbla23303（配列番号30），nbla23649，nbla24468（以上、新規遺伝子）、nbla20141，nbla20446，nbla21538，nbla21558，nbla21623，nbla21969，nbla22219，nbla23272，nbla23307およびnbla24117（以上、既知遺伝子）である。

【００５６】

III－３に属する特定のクローンは、nbla20578（配列番号26），nbla21212（以上、新規遺伝子）、nbla23478，nbla23896およびnbla24920（以上、既知遺伝子）である。

【００５７】

（グループIV）

このグループに属する遺伝子は、その発現（４ｓ）がＵＦと同程度であり、Ｆより高い（Ｆ＜４ｓ＝ＵＦ）。このグループに属する特定のクローンは、nbla23899（配列番号25）およびnbla24526（以上、新規遺伝子）である。

【００５８】

（グループV）

このグループに属する遺伝子は、その発現（４ｓ）がＦより低く、ＵＦより高い。さらに、これら遺伝子をサブグループに分類すると、Ｖ－１、Ｖ－２、Ｖ－３、Ｖ－４およびＶ－５となる。各サブグループの遺伝子発現パターンについては、表１を参照。

【０１５９】

Ｖ－１に属する特定のクローンは、nbla22031（既知）である。Ｖ－２に属する特定のクローンは、nbla22305（既知）である。

【００６０】

Ｖ－３に属する特定のクローンは、nbla20123（配列番号17），nbla20382（配列番号18），nbla20660（配列番号19），nbla20666（配列番号20），nbla21239（配列番号21），nbla21729（配列番号22），nbla21831（配列番号23），nbla22826（配列番号24），nbla24521（以上、新規遺伝子）、nbla20235およびnbla22607（以上、既知遺伝子）である。

【００６１】

Ｖ－４に属する特定のクローンは、nbla20787（配列番号15），nbla22284（配列番号16）およびnbla24756（以上、新規遺伝子）である。

【００６２】

Ｖ－５に属する特定のクローンは、nbla24348およびnbla24686（以上、新規遺伝子）である。

【００６３】

（グループVI）

このグループに属する遺伝子は、その発現（４ｓ）がＦおよびＵＦより低いか、またはＦおよびＵＦより高い。さらに、これら遺伝子をサブグループに分類すると、VI－１、VI－２、VI－３、VI－４、VI－５、VI－６、VI－７およびVI－８となる。各サブグループの遺伝子発現パターンについては、表１を参照。

【００６４】

VI－１に属する特定のクローンは、nbla21297（配列番号14）（新規遺伝子）およびnbla22443（既知遺伝子）である。

【００６５】

VI－２に属する特定のクローンは、nbla20211，nbla20469，nbla21250，nbla22182（配列番号12），nbla22761，nbla23256（配列番号13），nbla23631，nbla23711，nbla24532，nbla24951（以上、新規遺伝子）、nbla21750，nbla22129，nbla22808，nbla23064およびnbla23358（以上、既知遺伝子）である。

【００６６】

VI－３に属する特定のクローンは、nbla20226（配列番号11）（新規遺伝子）である。

【００６７】

VI－４に属する特定のクローンは、nbla21650（配列番号7），nbla22094（配列番号8），nbla22739（配列番号9）およびnbla23525（配列番号10）（以上、新規遺伝子）である。

【００６８】

VI－５に属する特定のクローンは、nbla23701（配列番号5）およびnbla23890（配列番号6）（以上、新規遺伝子）である。

【００６９】

VI－６に属する特定のクローンは、nbla20087（既知遺伝子）である。

【００７０】

VI－７に属する特定のクローンは、nbla22689（配列番号2），nbla22968，nbla24079，nbla24135（配列番号3）およびnbla24350（配列番号4）（以上、新規遺伝子）である。

【００７１】

VI－８に属する特定のクローンは、nbla22256（新規遺伝子）である。

【００７２】

（グループVII）

このグループに属する遺伝子（１個のみ）は、４ｓでのみ発現している。その特定のクローンは、nbla22420（配列番号1）（新規遺伝子）である。

【００７３】

前記それぞれのグループについて、遺伝子群を新規な遺伝子と、既知の遺伝子に分け、まとめたものが表１である。

【表1】

グループ	発現パターン	新規遺伝子	既知遺伝子	計
I-1	F≫4s=UF	5	0	5
I-2	F>4s=UF	59	16	75
I-3	F≧4s=UF	12	11	23
II-1	F=4s≫UF	18	5	23
II-2	F=4s>UF	105	47	152
II-3	F=4s≧UF	55	40	95
III-1	F=4s≪UF	1	1	2
III-2	F=4s<UF	10	10	20
III-3	F=4s<UF	2	3	5
IV	F<4s=UF	2	0	2
V-1	F>4s≫UF	0	1	1
V-2	F≧4s≫UF	0	1	1
V-3	F>4s>UF	9	2	11
V-4	F≧4s>UF	3	0	3
V-5	F≧4s≧UF	2	0	2
VI-1	F≫4s<UF	1	1	2
VI-2	F>4s<UF	10	5	15
VI-3	F>4s≦UF	1	0	1
VI-4	F≧4s≦UF	4	0	4
VI-5	F<4s≫UF	2	0	2
VI-6	F≦4s≫UF	0	1	1
VI-7	F<4s>UF	5	0	5
VI-8	F≦4s≧UF	1	0	1
VII	4s のみ	1	0	1
クローン総数		308	144	452

　　なお、表中および上記分類において、「＝」は遺伝子発現量がサブセット間で
ほぼ等しいことを示す。

【００７４】

　例えば、グループⅥに属する遺伝子群は、４ｓ期神経芽細胞腫における遺伝子発現量と、予後良好型および予後不良型の臨床組織における同一遺伝子の遺伝子発現量を比較すると、４ｓ期神経芽細胞腫において特異的である（すなわち、いずれよりもかなり高いか、或いはかなり低い）。従って、これらの遺伝子の少なくともひとつの存在を臨床組織サンプルに検出すれば、４ｓ期神経芽細胞腫である可能性が高いとの判定ができる。

【００７５】

　また、グループⅦに属する遺伝子は、４ｓ期神経芽細胞腫の臨床組織においてのみ、検出されている。従って、この遺伝子の存在を臨床組織サンプルに検出すれば、４ｓ期神経芽細胞腫である可能性が高いとの判定ができることになる。

【００７６】

　さらに、残りのグループに属する遺伝子群も、４ｓ期神経芽細胞腫における、遺伝子発現量と、予後良好型および予後不良型の臨床組織における同一遺伝子の遺伝子発現量を比較すると、上記のような発現パターンが見出される。従って、これらの遺伝子の発現パターンを複数個、検出して、それらを解析すれば、検定する臨床組織サンプルが４ｓ期神経芽細胞腫であるかどうかの判定ができる。特に、この目的で本発明の核酸を使用するとき、後述の核酸マイクロアレイを作製して、前記判定に供することが好ましい。

【００７７】

　このように、本発明の核酸は神経芽細胞腫の予後の良不良を診断する腫瘍マーカーとして有用である。すなわち、本発明は、ヒト神経芽細胞腫の予後およびそれに関連する様々な遺伝子情報を以下の手段により提供可能とする。

【００７８】

（１）ハイブリダイゼーションに用いるプローブ

　本発明の１つの実施の形態に従えば、本発明の核酸をハイブリダイゼーションのプローブ（すなわち、本発明の核酸プローブ）として使用することによって、神経芽細胞腫で発現している本発明の遺伝子を検出することが可能である。さらに、本発明の核酸をハイブリダイゼーションのプローブとして使用し、様々な腫

瘍、正常組織における遺伝子発現を調べることによって、該遺伝子発現の分布を同定することも可能である。

【００７９】

本発明の核酸をハイブリダイゼーションのプローブとして使用する場合、ハイブリダイゼーション方法自身については特に限定されない。好適な方法としては、例えばノザンハイブリダイゼーション、サザンハイブリダイゼーション、コロニーハイブリダイゼーション、ドットハイブリダイゼーション、Fluorescence in situ hybridization（ＦＩＳＨ）、in situ hybridization（ＩＳＨ）、ＤＮＡチップ法、マイクロアレイ法、などが挙げられる。

【００８０】

前記ハイブリダイゼーションの１つの応用例として、本発明の核酸をノザンハイブリダイゼーションのプローブとして用い、検定する臨床組織サンプル中においてｍＲＮＡの長さを測定することや、遺伝子発現を定量的に検出することが可能である。

【００８１】

また、別の応用例として、本発明の核酸をサザンハイブリダイゼーションのプローブとして用い、検定する臨床組織サンプルのゲノムＤＮＡ中の、該ＤＮＡ配列の有無を検出することが可能である。

【００８２】

さらに別の応用例として、本発明の核酸をＦＩＳＨ法のプローブとして用い、本発明の遺伝子の染色体上の位置を同定することも可能である。

【００８３】

さらに別の応用例として、本発明の核酸をＩＳＨ法のプローブとして用い、本発明の遺伝子の発現の組織分布を同定することも可能である。

【００８４】

本発明の核酸をハイブリダイゼーション用プローブとして使用する場合、少なくとも２０個の塩基長が必要であり、本発明の核酸のうち、２０個以上の連続した塩基からなる核酸が好ましく用いられる。より好ましくは、４０個以上の連続した塩基からなる核酸が用いられる。特に好ましくは、６０個以上の連続した塩

基からなる核酸が用いられる。さらに、配列表の配列番号１〜１７４に記載の核酸配列の全長からなる核酸を用いてもよい。

【０○８５】

当業者にとって、上記各種のハイブリダイゼーションにおける核酸プローブ技法は周知であり、例えば、個々の塩基長を有する本発明の核酸プローブと、目的とするポリヌクレオチドとの適当なハイブリダイズ条件は容易に決定することができる。種々の塩基長を含むプローブに対し至適であるハイブリダイズ条件を得るためのかかる操作は、当業者では周知であり、例えばサンブルックら、モレキュラー・クローニング：ア・ラボラトリー・マニュアル（Molecular Cloning: A laboratory manual）（前掲）を参照して、行えばよい。

【００８６】

好ましくは、本発明の核酸プローブは、容易に検出されるように標識される。検出可能な標識は、目視によって、または機器を用いるかのいずれかによって検出され得るいかなる種類、元素または化合物であってもよい。通常使用される検出可能な標識としては、放射性同位元素、アビジンまたはビオチン、蛍光物質（ＦＩＴＣまたはローダミン等）が挙げられる。前記放射性同位元素は、^{32}P、^{14}C、^{125}I、^{3}H、^{35}S等である。また、ビオチン標識ヌクレオチドは、ニックトランスレーション、化学的または酵素的手段によって、核酸に組み込むことができる。ビオチン標識されたプローブは、アビジン／ストレプトアビジン、蛍光標識、酵素、金コロイド複合体等などの標識手段を使用したハイブリダイゼーション後に検出される。また、本発明の核酸プローブは、タンパク質と結合させることによって標識されてもよい。その目的で、例えば放射性または蛍光ヒストン一本鎖結合タンパク質が使用される。このようにして、適当に標識されたプローブは、本発明の診断剤を構成する。

【００８７】

（２）ＰＣＲに用いるプライマー

本発明の遺伝子を検出するには上記のハイブリダイゼーション法の他に、本発明の核酸に含まれる任意の核酸（ＤＮＡ）配列からプライマーを設計して、Polymerase Chain Reaction（ＰＣＲ）法を用いることにより可能である。例えば、

検定する臨床組織サンプルからｍＲＮＡを抽出し、ＲＴ－ＰＣＲ法により遺伝子発現を半定量的に測定することが可能である。このような方法は、当業者にとって周知の方法に従って行われるが、例えば、サンブルックら、モレキュラー・クローニング：ア・ラボラトリー・マニュアル（Molecular Cloning: A laboratory manual）（前掲）、および遺伝子病入門（高久史麿著：南江堂）が参照される。

【００８８】

　本発明の核酸（ＤＮＡ）をＰＣＲ用プライマー（すなわち、本発明のプライマー）として使用する場合、１０ないし６０個の塩基長が必要であり、本発明に係る核酸配列の一部であって、１０ないし６０個の連続した塩基を有する核酸が好ましく用いられる。より好ましくは、１５ないし３０個の塩基を有するものが用いられる。また一般的には、プライマー配列中のＧＣ含量が４０ないし６０％のものが好ましい。さらに、増幅に用いる２つのプライマー間のＴｍ値に差がないことが望まれる。また、プライマーの３’末端でアニールせず、プライマー内で２次構造をとらないことも望ましい。

【００８９】

（３）遺伝子のスクリーニング

　本発明の核酸を使用することによって、神経芽細胞腫のみならず様々な組織や細胞で発現している本発明の遺伝子の発現（またはその分布）を検出することが可能である。これは例えば、本発明の核酸を上記のようにハイブリダイゼーションのプローブ、またはＰＣＲのプライマーとして使用することによって、可能となる。

【００９０】

　また、ＤＮＡチップ、核酸マイクロアレイ等を用いても遺伝子の発現分布を検出することが可能である。すなわち、本発明の核酸を直接、前記チップ、アレイ上に張り付けることが出来る。チップ、アレイに張り付けるために、高精度分注機でかかる核酸等（ＤＮＡ）を基板にスポットする方法が知られている（例えば、米国特許第５８０７５２２号を参照）。そこに臨床組織サンプルから抽出したｍＲＮＡを蛍光物質などで標識し、ハイブリダイズさせ、その遺伝子がどの様な

組織の細胞で高発現しているかを解析することが可能である。またチップ、アレイ上に張り付けるＤＮＡは、本発明の核酸またはその断片をプローブとして用いたＰＣＲの反応産物であってもよい。別法として、本発明の核酸断片（ＤＮＡ断片）を基板上で直接合成してＤＮＡチップ若しくはアレイとすることもできる（例えば、米国特許第５２４１８６号を参照）。

【００９１】

（４）ＤＮＡのクローニング

　本発明の核酸を使用することによってヒト神経芽細胞腫において発現している遺伝子をクローニングすることが可能である。例えば、本発明の核酸をノザンハイブリダイゼーションのプローブ、コロニーハイブリダイゼーションのプローブまたはＰＣＲのプライマーとして使用し、本発明の遺伝子をクローニングすることが可能である。クローニング可能な遺伝子としては特に、予後不良型の神経芽細胞腫と予後不良型の神経芽細胞腫で発現量に差がある遺伝子、４ｓ期神経芽細胞腫で発現する遺伝子、他の組織や癌細胞での発現様式とは異なって発現している遺伝子、細胞周期依存的に発現している遺伝子、神経分化に伴って誘導される遺伝子、癌遺伝子または癌抑制遺伝子によって発現が制御される遺伝子等が挙げられる。

【００９２】

（５）腫瘍の予後診断の方法およびそのために使用可能な腫瘍マーカー

　上述のように本発明の遺伝子は、４ｓ期神経芽細胞腫（予後良好型および予後不良型の神経芽細胞腫を含めて）において発現が見出された。そこで、本発明の核酸をハイブリダイゼーションのプローブ或いはＰＣＲのプライマーとして使用し、被験者から採取した、検定する臨床組織サンプル中で、前記遺伝子の発現パターンを調べることにより予後診断（４ｓ期神経芽細胞腫の判定）が行える。遺伝子の検出方法としては、前述のノーザンブロットハイブリダイゼーション法、インサイチュハイブリダイゼーション法、およびＲＴ－ＰＣＲ法等が挙げられる。

【００９３】

ハイブリダイゼーション法を用いるとき、検出する臨床組織サンプル中で前記

核酸プローブとハイブリダイズする核酸の量を対照サンプル（例えば、予後良好型および予後不良型の神経芽細胞腫からの臨床組織）と比較して、遺伝子発現パターンを決定する。このようにして遺伝子発現パターンを検出するのに使用したそれぞれの核酸について、例えば、表１に記載の発現パターンと比較、解析して、予後診断できる。この目的では、前記の核酸マイクロアレイの使用が望ましい。また、ＲＴ−ＰＣＲ法を用いるとき、サンプルからｍＲＮＡを抽出し、これをＤＮＡに逆転写して、前記プライマーにより増幅するＲＴ−ＰＣＲ法を用いて、遺伝子発現を半定量的に測定する。それから前記と同様にして、予後診断できる。この目的のためには、該プライマーを必須成分として一組含有する診断キットを用いることが好ましい。該診断キットは、プライマー成分以外に、ＰＣＲ用の緩衝液、洗浄液、および酵素等の公知の成分を含む。

【００９４】

（６）アンチセンスオリゴヌクレオチド

　本発明の別の実施の形態に従えば、本発明の核酸に対するアンチセンスオリゴヌクレオチドが提供される。前記アンチセンスオリゴヌクレオチドは、本発明の核酸にハイブリダイズすることが可能であり、アンチセンスＤＮＡとアンチセンスＲＮＡとを含む。アンチセンスＤＮＡは、ＤＮＡからｍＲＮＡへの転写を阻害し、アンチセンスＲＮＡは、ｍＲＮＡの翻訳を阻害する。このようなアンチセンスオリゴヌクレオチドは、自動合成機を使用して、または本発明の核酸を鋳型とするＰＣＲ法により合成できる。さらに、該アンチセンスオリゴヌクレオチドは、ＤＮＡやｍＲＮＡとの結合力、組織選択性、細胞透過性、ヌクレアーゼ耐性、細胞内安定性が高められたアンチセンスオリゴヌクレオチド誘導体をも包含する。このような誘導体は、公知のアンチセンス技術を用いて、合成することができる。

【００９５】

　ｍＲＮＡの翻訳開始コドン付近、リボソーム結合部位、キャッピング部位、スプライス部位の配列に相補的な配列を有するアンチセンスオリゴヌクレオチドは、該ＲＮＡの合成を阻止することができ、特に遺伝子の発現抑制効果が高い。従って、本発明は、かかるアンチセンスオリゴヌクレオチドを好適に包含する。

【００９６】

（7）遺伝子治療

　本発明の別の実施の形態に従えば、遺伝子治療に用いられる治療用遺伝子をコードする核酸配列が提供される。そこで、本発明の核酸を遺伝子運搬に使用されるベクターに導入して、任意の発現プロモーターにより導入遺伝子（本発明の遺伝子）を発現させ、遺伝子治療に用いることができる。

【００９７】

　1．ベクター

　導入されうるウイルスベクターは、ＤＮＡまたはＲＮＡウイルスをもとに作製できる。このようなベクターは、ＭｏＭＬＶベクター、ヘルペスウイルスベクター、アデノウイルスベクター、ＡＡＶベクター、ＨＩＶベクター、ＳＩＶベクター、センダイウイルスベクター等のいかなるウイルスベクターであってもよい。また、ウイルスベクターの構成タンパク質群のうち１つ以上を、異種ウイルスの構成タンパク質に置換する、または、遺伝子情報を構成する核酸配列のうち一部を異種ウイルスの核酸配列に置換する、シュードタイプ型のウイルスベクターも本発明に使用できる。例えば、ＨＩＶの外皮タンパク質であるＥｎｖタンパク質を、小水疱性口内炎ウイルス（vesicular stomatitis Virus: VSV）の外皮タンパク質であるＶＳＶ－Ｇタンパク質に置換したシュードタイプウイルスベクターが挙げられる［ナルジニ・エルら（Naldini L.），サイエンス（Science），米国，１９９６年，第２７２巻，p．２６３）］。さらに、治療効果を持つウイルスであれば、ヒト以外の宿主域を持つウイルスもウイルスベクターとして使用可能である。ウイルス以外のベクターとしてはリン酸カルシウムと核酸の複合体、リポソーム、カチオン脂質複合体、センダイウイルスリポソーム、ポリカチオンを主鎖とする高分子キャリアー等が使用可能である。さらに遺伝子導入系としてはエレクトロポレーション、遺伝子銃等も使用可能である。

【００９８】

　2．発現プロモーター

　さらに、治療用遺伝子に用いられる発現カセットは、標的細胞内で遺伝子を発現させることができるものであれば、特に制限されることなくいかなるものでも

用いることができる。当業者はそのような発現カセットを容易に選択することができる。好ましくは、動物由来の細胞内で遺伝子発現が可能な発現カセットであり、より好ましくは、哺乳類由来の細胞内で遺伝子発現が可能な発現カセットであり、特に好ましくは、ヒト由来の細胞内で遺伝子発現が可能な発現カセットである。発現カセットに用いられる遺伝子プロモーターは、例えばアデノウイルス、サイトメガロウイルス、ヒト免疫不全ウイルス、シミアンウイルス４０、ラウス肉腫ウイルス、単純ヘルペスウイルス、マウス白血病ウイルス、シンビスウイルス、Ａ型肝炎ウイルス、Ｂ型肝炎ウイルス、Ｃ型肝炎ウイルス、パピローマウイルス、ヒトＴ細胞白血病ウイルス、インフルエンザウイルス、日本脳炎ウイルス、ＪＣウイルス、パルボウイルスＢ１９、ポリオウイルス等のウイルス由来のプロモーター、アルブミン、ＳＲα、熱ショック蛋白、エロンゲーション因子等の哺乳類由来のプロモーター、ＣＡＧプロモーター等のキメラ型プロモーター、テトラサイクリン、ステロイド等によって発現が誘導されるプロモーターを含む。

【０９９】

３．医薬品

　遺伝子治療に用いる医薬品は、上記のような治療用にデザインされた薬物遺伝子を含む組換えウイルスベクターとして調製される。より具体的に言えば、本発明の遺伝子を含む組換えウイルスベクターを、水、生理食塩水、等張化した緩衝液等の適当な溶媒に溶解することで調製できる。その際、ポリエチレングリコール、グルコース、各種アミノ酸、コラーゲン、アルブミン等を保護材として添加しても調製可能である。

【０１００】

４．投与法、投与量

　上記医薬品の生体への投与の方法については特に制限はない。例えば非経口的投与（注射投与など）することにより好ましく実施できる。その医薬品の使用量は、その使用方法、使用目的等により異なり、当業者は容易に適宜選択および最適化することが可能である。例えば、注射投与して用いる場合には、１日量約０．１μｇ／ｋｇ〜１，０００ｍｇ／ｋｇを投与するのが好ましく、より好ましく

は、１日量約１μg／ｋｇ〜１００mg／ｋｇである。

【０１０１】

以下、実施例に即してさらに詳しく説明するが、本発明の技術的範囲はこれらの例に限定されるものではない。

【０１０２】

【実施例】

以下、実施例に基づいて本発明をより具体的に説明するが、本発明は以下の実施例に限定されるものではない。

【０１０３】

（製造例１）神経芽細胞腫からのｃＤＮＡライブラリーの作製

１．サンプル入手

ヒト神経芽細胞腫（４ｓ期）の臨床組織サンプルを手術摘出直後に準無菌的に凍結し、その後−８０℃に保存した。

【０１０４】

２．ｍＲＮＡの調製

１に記載のサンプル２〜３ｇをTotal RNA Extraction Kit（QIGEN社製）で処理し、トータルＲＮＡを抽出した。抽出したトータルＲＮＡをオリゴｄＴセルロースカラム（Collaborative社製）を用いて、ｐｏｌｙ　Ａ構造を有するｍＲＮＡプールに精製した。さらに、以下の手順に従い、オリゴキャッピング法［Y．Suzukiら、ジーン（Gene），米国，１９９７年，第２００巻，ｐ．１４９−１５６］を用いてｃＤＮＡライブラリーを調製した。

【０１０５】

３．ｍＲＮＡの脱リン酸化

上記２において調製した１００〜２００μgのｍＲＮＡプールを６７．３μｌの０．１％ジエチルピロカーボネート（ＤＥＰＣ）を含む滅菌超純水（ＤＥＰＣ−Ｈ２Ｏ）に溶解させ、２０μｌの５ｘＢＡＰバッファー［Ｔｒｉｓ−ＨＣｌ（５００mM、ｐＨ＝７．０）／メルカプトエタノール（５０mM）］、２．７μｌのＲＮａｓｉｎ（４０ｕｎｉｔ／μｌ：Ｐｒｏｍｅｇａ社製）、１０μｌのＢＡＰ（０．２５ｕｎｉｔ／μｌ、バクテリア由来アルカリフォスファターゼ：宝

酒造社製）を加えた。この混合液を３７℃で１時間反応させ、ｍＲＮＡの５'末端の脱リン酸化処理を行った。その後、フェノール・クロロホルム処理を２回行い、最後にエタノール沈殿により、脱リン酸化ｍＲＮＡプールを精製した。

【０１０６】

４．脱リン酸化ｍＲＮＡの脱キャップ処理

上記３において調製した脱リン酸化ｍＲＮＡプールの全量を７５．３μｌの０．１％ＤＥＰＣを含む滅菌超純水に溶解させ、２０μｌの５ｘＴＡＰバッファー［酢酸ナトリウム（２５０ｍＭ、ｐＨ＝５．５）／メルカプトエタノール（５０ｍＭ）、ＥＤＴＡ（５ｍＭ、ｐＨ＝８．０）］、２．７μｌのＲＮａｓｉｎ（４０ｕｎｉｔ／μｌ）、２μｌのＴＡＰ（Tobacco Acid pyrophosphatase：２０ｕｎｉｔ／μｌ）］を加えた。この混合液を３７℃で１時間反応させ、脱リン酸化ｍＲＮＡの５'末端の脱キャップ処理を行った。この際、キャップ構造を持たない不完全長の脱リン酸化ｍＲＮＡは、脱キャップ処理されず５'末端は脱リン酸化された状態に留まった。その後、フェノール・クロロホルム処理、エタノール沈殿により、脱キャップｍＲＮＡプールを精製した。

【０１０７】

５．オリゴキャップｍＲＮＡの調製

上記４において調製した脱キャップｍＲＮＡプールの全量を１１μｌの０．１％ＤＥＰＣを含む滅菌超純水に溶解させ、４μｌの５'－オリゴＲＮＡ（５'－ＡＧＣＡＵＣＧＡＧＵＣＧＧＣＣＵＵＧＧＣＣＵＡＣＵＧＧ－３'：配列番号１０７９；１００ｎｇ／μｌ）、１０μｌの１０ｘｌｉｇａｔｉｏｎバッファー［Ｔｒｉｓ－ＨＣｌ（５００ｍＭ、ｐＨ＝７．０）／メルカプトエタノール（１００ｍＭ）］、１０μｌの塩化マグネシウム（５０ｍＭ）、２．５μｌのＡＴＰ（２４ｍＭ）、２．５μｌのＲＮａｓｉｎ（４０ｕｎｉｔ／μｌ）、１０μｌのＴ４　ＲＮＡ　ｌｉｇａｓｅ（２５ｕｎｉｔ／μｌ：宝酒造社製）、５０μｌのポリエチレングリコール（５０％ｗ／ｖ、ＰＥＧ８０００：シグマ社製）を加えた。この混合液を２０℃で３時間反応させ、脱キャップｍＲＮＡの５'末端に５'－オリゴＲＮＡを連結した。この際、キャップ構造を持たない不完全長の脱リン酸化ｍＲＮＡは、５'－オリゴＲＮＡが連結されない。その後、フェノール・クロロホルム処理、エタノール沈殿に

より、オリゴキャップｍＲＮＡプールを精製した。

【０１０８】

６．オリゴキャップｍＲＮＡからのＤＮＡ除去

上記５において調製したオリゴキャップｍＲＮＡプールを７０．３μｌの０．１％ＤＥＰＣを含む滅菌超純水に溶解させ、４μｌのＴｒｉｓ－ＨＣｌ（１Ｍ、ｐＨ＝７．０）、５．０μｌのＤＴＴ（０．１Ｍ）、１６μｌの塩化マグネシウム（５０ｍＭ）、２．７μｌのＲＮａｓｉｎ（４０ｕｎｉｔ／μｌ）、２μｌのＤＮａｓｅＩ（５ｕｎｉｔ／μｌ：宝酒造社製）を加えた。この混合液を３７℃で１０分間反応させ、余分なＤＮＡを分解した。その後、フェノール・クロロホルム処理、エタノール沈殿、カラム精製（Ｓ－４００ＨＲ：ファルマシアバイオテック社製）により、ＤＮＡ（－）オリゴキャップｍＲＮＡプールを精製した。

【０１０９】

７．First Strand　ｃＤＮＡの調製

上記６において調製したＤＮＡ（－）オリゴキャップｍＲＮＡプールを、Super Script II（ライフテックオリエンタル社製キット）を用いて逆転写し、First Strand　ｃＤＮＡプールを得た。

【０１１０】

ＤＮＡ（－）オリゴキャップｍＲＮＡプールを２１μｌの滅菌蒸留水に溶解させ、１０μｌの１０ｘFirst strandバッファー（キット付属品）、８μｌのｄＮＴＰｍｉｘ（５ｍＭ、キット付属品）、６μｌのＤＴＴ（０．１Ｍ、キット付属品）、２．５μｌのオリゴ－ｄＴアダプタープライマー（５ｐｍｏｌ／μｌ、５'-GCGGCTGAAGACGGCCTATGTGGCCTTTTTTTTTTTTTTTTTT－３'配列番号１０８０）、２．０μｌのＲＮａｓｉｎ（４０ｕｎｉｔ／μｌ）、２μｌのSuper Script II RTase（キット付属品）を加えた。この混合液を４２℃で３時間反応させ、逆転写反応を行った。その後、フェノール・クロロホルム処理、アルカリ処理、中和処理にて全てのＲＮＡを分解し、エタノール沈殿で精製した。

【０１１１】

８．Second Strand　ｃＤＮＡの調製

上記７において調製したFirst Strand　ｃＤＮＡプールを、Ｇｅｎｅ　Ａｍｐ

（パーキンエルマー社製キット）を用いて、ＰＣＲ増幅した。First Strand　c
DNAプールを５２．４μlの滅菌蒸留水に溶解させ、３０μlの３．３ｘＲe
actionバッファー（キット付属品）、８μlのdNTP　mix（２．５
mM、キット付属品）、４．４μlの酢酸マグネシウム（２５mM、キット付属
品）、１．６μlのプライマーＦ（１０ｐｍｏｌ／μl、５’−AGCATCGAGTCGGCC
TTGTTG−３’配列番号１０８１）、１．６μlのプライマーＲ（１０ｐｍｏｌ／
μl、５’−GCGCTGAAGACGGCCTATGT−３’配列番号１０８２）、２μlのrTth
（キット付属品）を加えた。この混合液に、１００μlのミネラルオイルを静か
に加え重層した。この反応液を９４℃で５分間変性させた後、９４℃、１分間、
５２℃、１分間、７２℃、１０分間を１サイクルとして１２サイクル繰り返し、
さらに７２℃で１０分間放置し、ＰＣＲ反応を行った。その後、フェノール・ク
ロロホルム処理、エタノール沈殿で精製し、Second Strand　cDNAプールを
得た。

９．Second Strand　cDNAのSfiI処理

　上記８において調製したSecond Strand　cDNAプールを８７μlの滅菌蒸
留水に溶解させ、１０ｘＮＥＢバッファー（ＮＥＢ社製）、１００ｘＢＳＡ（ウ
シ血清アルブミン、ＮＥＢ社製）、２μlのSfiI（制限酵素、２０ｕｎｉｔ
／μl、ＮＥＢ社製）を加えた。この混合液を５０℃で一晩反応させ、SfiI
による制限酵素処理を行った。その後、フェノール・クロロホルム処理、エタノ
ール沈殿で精製し、両末端がSfiI処理されたcDNAプールを得た。

　【０１１２】

　１０．SfiI処理されたcDNAのサイズ分画

　上記９において調製したSfiI処理されたcDNAプールを１％のアガロー
スゲルで電気泳動し、２ｋｂ以上の分画をGene clean II（Ｂｉｏ　１０１社製
）を用いて精製した。精製したcDNAプールは１００μlの滅菌蒸留水に溶解
させ、３７℃で６時間放置した。その後、フェノール・クロロホルム処理、エタ
ノール沈殿で精製し、長鎖cDNAプールを得た。

　【０１１３】

　１１．cDNAライブラリー

　　上記１０において調製した長鎖ｃＤＮＡプールをDNA Ligation kit ver.1（宝酒造社製キット）を用いてクローニングベクターであるｐＭＥ１８Ｓ―ＦＬ３（東京大学医科学研究所　菅野純夫教授より供与）にライゲーションを行った。長鎖ｃＤＮＡプールを８μｌの滅菌蒸留水に溶解し、あらかじめ制限酵素ＤｒａＩＩＩで処理した１μｌのｐＭＥ１８Ｓ―ＦＬ３、８０μｌのＳｏｌｕｔｉｏｎＡ（キット付属品）、１０μｌのＳｏｌｕｔｉｏｎ　Ｂ（キット付属品）を加え、１６℃で３時間反応させた。その後、フェノール・クロロホルム処理、エタノール沈殿で精製しｃＤＮＡライブラリーを得た。

【０１１４】

（実施例１）大腸菌へのトランスフォーメーション

１．クローニング

　　製造例１の１２で調製したｃＤＮＡライブラリーを大腸菌（ＴＯＰ－１０、Invitrogen社製）にトランスフォーメーションした。すなわち、ｃＤＮＡライブラリーを１０μｌの滅菌蒸留水に溶解し、ＴＯＰ－１０に混合した。その後、氷上にて３０分間、４０℃で１分間、氷上で５分間インキュベートした。５００μｌのＳＯＢ培地を加え、３７℃で６０分間振盪培養した。アンピシリンを含む寒天培地上に適量づつ播種し、３７℃で一昼夜培養して、大腸菌クローンを得た。ここで、５０７５個のクローンを無作為にピックアップした。

【０１１５】

２．大腸菌クローンの保存（グリセロールストックの調製）

　　上記１において得られた寒天培地上の各大腸菌クローンを、爪楊枝にて拾い上げ、９６穴プレートに準備した１２０μｌのＬＢ培地中に懸濁させた。この９６穴プレートを３７℃で一晩静置し、大腸菌の培養を行った。その後、６０％グリセロール溶液を７２μｌ加え、－２０℃で保存した（グリセロールストック）。

【０１１６】

（実施例２）核酸配列決定

１．プラスミドの調製

　　実施例１の２で調製した１０μｌのグリセロールストックを１５ｍｌの遠心チューブに移し、３ｍｌのＬＢ培地、５０μｇ／ｍｌのアンピシリンを加え、３７

℃で一晩振盪し、大腸菌の培養を行った。その後、QIA Prep Spin Miniprep Kit（QIAGEN社製）を用いて大腸菌からプラスミドＤＮＡを抽出、精製した。

【０１１７】

２．両末端シークエンスの解析

上記１において調製したプラスミドＤＮＡをDNA Sequencing Kit（ＡＢＩ社製キット）を用いて両末端のシークエンスを決定した。６００ｎｇのプラスミドＤＮＡ、８μｌのプレミックス（キット付属品）、３．２ｐｍｏｌのプライマーを混合し、滅菌蒸留水で合計２０μｌになるように調製した。この混合液を９６℃で２分間変性させた後、９６℃、１０秒間、５０℃、５秒間、６０℃、４分間を１サイクルとして２５サイクル繰り返し反応を行った。その後エタノール沈殿で精製した。変性条件下でポリアクリルアミドゲルにて電気泳動を行い、ＡＢＩ３７７（ＡＢＩ社製）を用いて配列決定を行った。

【０１１８】

（実施例３）データベースを用いるホモロジー検索

実施例２において両末端シークエンスを解析して得られたサンプルのＤＮＡ配列情報についてインターネットを介したＤＮＡ配列のホモロジー検索を行った。検索にはＮＣＢＩ（National Center of Biotechnology Information USA, http://www.ncbi.nlm.nih.gov/BLAST）のＢＬＡＳＴを用いた。ＢＬＡＳＴサーチのソフトとして、DYNACLUST Ver.4.0（DYNACOM社）を使用した。ホモロジー検索の結果、約２７００個の遺伝子を同定した。これらの遺伝子を分類し、RepeatMaskerソフトを使用して反復配列を取り除いたところ、１５９８個の遺伝子が得られた。そのうち、新規な遺伝子は、９６３個であり、既知の遺伝子は６３５個であった。

【０１１９】

これらの遺伝子のうち、新規なもの３０８個については、シークエンスできたもの関して、配列表にそれらの部分解読配列を示してある。

【０１２０】

（実施例４）半定量的ＲＴ－ＰＣＲによる遺伝子発現の比較

１．サンプル入手

　　ヒト神経芽細胞腫（４ｓ期）の臨床組織サンプルを手術摘出直後に準無菌的に凍結し、その後－８０℃に保存した。このようなサンプルを８検体用意した。同様に、予後良好型および予後不良型のヒト神経芽細胞腫の臨床組織サンプルを各１２検体づつ用意した。

【０１２１】

　　予後良好型および予後不良型の神経芽細胞腫サンプルについては、予後の検定を以下の指標をもとに行ったものである。

　　予後良好型：

　　・病期１または２

　　・発症年齢が１歳未満

　　・手術後５年以上再発なく生存

　　・N-mycの増幅なし

　　予後不良型：

　　・病期４

　　・発症年齢が１歳以上

　　・手術後３年以内に死亡

　　・N-myc増幅あり

【０１２２】

　　２．ディファレンシャルスクリーニング

　　各検体の半定量的ＲＴ－ＰＣＲは以下の方法により実施した。

ａ）逆転写（ＲＴ）反応

【０１２３】

　　検体からのＲＮＡをSuperScript II reverse transcriptase（ＧＩＢＣＯ社製）を用いて、ｃＤＮＡに逆転写した。すなわち、トータルＲＮＡ２０μｇ、８μｌのランダムプライマー（１μｇ／μｌ）（宝酒造社製）、および必要量のＤＥＰＣを含む滅菌超純水で４８μｌの溶液を調製した。この溶液を６５℃で１５分間、インキュベートし、反応終了後氷上に置いた。２４μｌの５ｘFirst Strand Buffer（ＧＩＢＣＯ社製）、１２μｌの０．１Ｍ　ＤＴＴ（ＧＩＢＣＯ社製）、３０μｌのｄＮＴＰｓ（宝酒造社製）、４μｌのSuper Script II reverse tr

anscriptase、および２μｌのＤＥＰＣを含む滅菌超純水を混合して、７２μｌの混合液を調製した。この混合液を前記の氷冷した溶液に加え、総量を１２０μｌとし、４２℃で１．５時間、次いで９５℃で５分間反応させた。これを－２０℃で保存し、ＰＣＲ鋳型の母液とした。

【０１２４】

このように調製したｃＤＮＡ溶液をＤＤＷで適当な倍率に希釈し、ＧＡＰＤＨプライマーを用いて、標準化（濃度調整）した。使用したＧＡＰＤＨプライマーの塩基配列は、下記の通りであった。

5'-ACCTGACCTGCCGTCTAGAA-3'（forward:配列番号１０７７）

5'-TCCACCACCCTGTTGCTGTA-3'（reverse:配列番号１０７８）

【０１２５】

続いて、ＤＤＷで希釈、濃度調整した各サンプルを下記のＰＣＲ反応に供した。

ｂ）ＰＣＲ反応

【０１２６】

ＰＣＲ反応は、rTaq polymerase（宝酒造社製）を用いて行った。前記４ｓ期神経芽細胞腫からのｃＤＮＡライブラリーで同定された（新規或いは既知を問わず）遺伝子に対して、適当なプライマーを設計し、濃度調整した３組のｃＤＮＡサンプル集団のディファレンシャルスクリーニングを行った。すなわち、２μｌのｃＤＮＡ、５μｌの滅菌蒸留水、１μｌの１０ｘｒＴａｑバッファー、１μｌの２ｍＭ　ｄＮＴＰｓ、各々０．５μｌの合成プライマーセット（forwardおよびreverse）、０．５μｌのｒＴａｑを混合した。この混合液を９５℃で２分間変性させた後、９５℃、１５秒間、５８℃、１５秒間、７２℃、２０秒間を１サイクルとして３５サイクル繰り返し、さらに７２℃で２０分間放置し、ＰＣＲ反応を行った。使用するプライマーセットによって、バンドが現れなかった場合、サイクル数を増加して、ＰＣＲ条件を検討し、それぞれのプライマーのアニーリング温度とサイクル数を決定できた。

【０１２７】

このように設定した条件でＰＣＲを行った産物を１．５％アガロースゲルで２

０分間電気泳動し、エチジウムブロミドで染色して、３組の検体（４ｓ期神経芽細胞腫、予後良好型の神経芽細胞腫、および予後不良型の神経芽細胞腫）におけるバンドの濃度を比較した。

【０１２８】

得られた発現パターンを検体サブセット間で、まとめたものが既出の表１である。また、発現パターンの解析の結果は、既に議論した通りである。

【０１２９】

なお、使用したプライマーは、検出しようとする遺伝子の末端シークエンス（実施例３）をPrimer3ソフトに入力して、適当なプライマー選択条件（塩基数、Ｔｍ、ＧＣ％）で選定した。前出の特定クローンに対応するプライマー配列は、配列表（配列番号１７５～１０７６）に与えられている。

【０１３０】

【発明の効果】

以上説明したように、本発明の遺伝子または本発明の核酸から得られる情報を利用することにより、検定する臨床組織サンプルから該遺伝子を検出して、神経芽細胞腫の予後診断（主に４ｓ期神経芽細胞腫の判定）が可能となる。具体的には、前記遺伝子若しくは核酸から得られる情報を腫瘍マーカーに利用することにより、予後診断に使用可能な、診断剤の調製或いは診断用核酸マイクロアレイを設計することが可能となる。

【０１３１】

４ｓ期神経芽細胞腫の正しい診断ができれば、対象患者に治療が必要かどうかの判断の重要な情報となり、場合によれば不必要な外科手術を避けることができる。

【配列表】

SEQUENCE LISTING

<110> Hisamitsu Pharmaceutical Co., Inc.

<120> Nucleic acids isolated from stage 4s neuroblastoma

<130> JP02-1246-HM

<160> 1082

<170> PatentIn Ver. 2.1

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<213> Homo sapiens

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<400> 92

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<223> nbla22119

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<213> Homo sapiens

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<400> 96

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<210> 849

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<223> Synthetic Primer: nbla21067-1f(k)

<400> 849

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<210> 850

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<400> 853

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<400> 861

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<210> 862

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<210> 863

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<210> 871

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<223> Synthetic Primer: nbla22548-1f(k)

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<210> 883

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<400> 883
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<210> 886

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<211> 20

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<210> 889

<211> 19

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<210> 891

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<223> Synthetic Primer: nbla23556-1r

<400> 892

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<210> 894

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<223> Synthetic Primer: nbla24098r1-1f(k)

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<400> 907

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<223> Synthetic Primer: nbla24958-1f(k)

<400> 909

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<400> 913

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<223> Synthetic Primer: nbla20423d-1r

<400> 916

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<210> 917

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<223> Synthetic Primer: nbla20510-1f(k)

<400> 917

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<210> 918

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<400> 918

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<223> Synthetic Primer: nbla20833d-lf(k)

<400> 919

gctaatcaaa gcggcaaca   19

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<223> Synthetic Primer: nbla21268-1f(k)

<400> 927

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<210> 928

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<223> Synthetic Primer: nbla21273-1r

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<223> Synthetic Primer: nbla21614-1f(k)

<400> 935

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<223> Synthetic Primer: nbla21614-1r

<400> 936

ctgtaaacca tgaagatgca ga   22

<210> 937

<211> 21

<212> DNA

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<223> Synthetic Primer: nbla21624-1f(k)

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<210> 938

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<223> Synthetic Primer: nbla21655-1f(k)

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<223> Synthetic Primer: nbla21954-1r

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<210> 947

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<210> 949

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<223> Synthetic Primer: nbla22043-1r

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<223> Synthetic Primer: nbla22137rl-lf(k)

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aggcattaag ggcacacc   18

<210> 952

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<400> 975

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<223> Synthetic Primer: nbla23683-1f(k)

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<223> Synthetic Primer: nbla23823-1f(k)

<400> 991

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<223> Synthetic Primer: nbla23823-1r

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<223> Synthetic Primer: nbla23849-1f(k)

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<223> Synthetic Primer: nbla23849-1r

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<223> Synthetic Primer: nbla23882-1f(k)

<400> 995

tgctactggg agctgatgtg   20

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<210> 997

<211> 21

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<400> 997

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<210> 998

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<223> Synthetic Primer: nbla23910rl-1r

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<210> 999

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<223> Synthetic Primer: nbla24064-1f(k)

<400> 999

cggaggagaa acggaggt   18

<210> 1000

<211> 19

<212> DNA

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<223> Synthetic Primer: nbla24064-1r

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<210> 1001

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<223> Synthetic Primer: nbla24405-1f(k)

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<400> 1002

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<210> 1003
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<400> 1003

aggagtttgc tgctgctctc   20

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<223> Synthetic Primer: nbla20624d-1f(k)

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<210> 1008

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<400> 1009

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<223> Synthetic Primer: nbla22029-1r

<400> 1010

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<223> Synthetic Primer: nbla22424r1d-1f(k)

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<400> 1012

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<210> 1013

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<400> 1013

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<400> 1015

gcaaggggt cttcttcct   19

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<223> Synthetic Primer: nbla22622-1r

<400> 1016

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<210> 1017

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<400> 1021

acgtggattt atggtctgtg g   21

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<210> 1040

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<400> 1045

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<210> 1046

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<223> Synthetic Primer: nbla21168-1r

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<223> Synthetic Primer: nbla21303-1r

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<223> Synthetic Primer: nbla21917-1f(k)

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<223> Synthetic Primer: nbla22099-1f(k)

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<223> Synthetic Primer: nbla22438-1f(k)

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<223> Synthetic Primer: nbla24118-1f(k)

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<210> 1067

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acatcaac ttccctccaa　　　　　　　　　　　　　　　　　　　20..

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<223> Synthetic Primer: GAPD forward

<400> 1077

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<211> 20

<212> DNA

<213> Artificial Sequence

<220>

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<210> 1080

<211> 42

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<400> 1080

gcggctgaag acggcctatg tggccttttt ttttttttt tt   42

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<220>

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agcatcgagt cggccttgtt g   21

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gcgctgaaga cggcctatgt   20

出証特２００３－３１００１６７

【書類名】　　要約書

【要約】

【課題】　　４ｓ期神経芽細胞腫に特徴的な遺伝子を同定し、それら遺伝子の核酸配列情報に基づき、神経芽細胞腫の予後（特に、進行度分類および４ｓ期神経芽細胞腫の判定）を診断する。

【解決手段】　　配列表の配列番号１ないし１７４に記載の核酸配列からなる群より選ばれる１つの配列からなる核酸若しくはその断片等、或いはその組み合わせを利用した核酸プローブ、プライマーまたは核酸マイクロアレイからなる、神経神経芽細胞腫の予後診断剤および診断キットを用いて、神経芽細胞腫の予後（特に、進行度分類および４ｓ期神経芽細胞腫の判定）を診断する。

【選択図】　　なし

特願２００２－３１６５８６

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